Genetic Evidence Against Intramolecular Rejoining of the Donor DNA Molecule Following IS*10* Transposition

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ABSTRACT

Tn*10* and IS*10* transpose by a nonreplicative mechanism in which the transposon is excised from the donor molecule and integrated into a target DNA site, leaving behind a break at the original donor site. The fate of this broken donor DNA molecule is not known. We describe here two experiments that address this issue. One experiment demonstrates that a polar IS*10* element gives rise to polarity-relief revertants at less than 1% the frequency of transposition of the same element in the same culture. In a second experiment, transpositions of an IS*10* element from one site in the bacterial genome to another are selected and the resulting isolates examined for alterations at the donor site; none of 1088 such isolates exhibited a detectable change at the donor locus. These results are compatible with two possible fates of the transposon donor molecule: degradation ("donor suicide"), or restoration of the original information at the donor site by a recombinational repair mechanism analogous to double-strand break repair. These results argue against the possibility that the donor molecule gap is simply resealed by intramolecular rejoining.

MANY lines of evidence suggest that the bacterial transposon Tn*10* and its component insertion sequence IS*10* transpose by a nonreplicative mechanism in which the element is excised from its donor site by double-strand breaks at its ends and then inserted into a new target site (BENDER and KLECKNER 1986; BENJAMIN and KLECKNER 1989; HANIFORD, BENJAMIN and KLECKNER 1991). An important unsolved mechanistic and biological issue is the fate of the transposon donor molecule.

Several possible fates can be imagined (BENDER and KLECKNER 1986; Figure 1). First, the donor molecule might remain broken, and eventually be degraded and lost. We will refer to this fate as donor suicide. Second, the broken donor molecule might be repaired. In this case, the repair process might regenerate transposon sequences at the donor site, presumably by a recombinational repair mechanism analogous to double strand break repair in which a sister chromosome serves as a template for resynthesis of the excised information (SZOSTAK *et al.* 1983; THALER and STAHL 1988). We will refer to this type of mechanism as gap restoration. Alternatively, the repair process might occur in such a way that the original transposon sequences are not restored, with or without additional degradation around the excision site. For example, the two ends of the donor molecule might simply be religated. We will refer to this general type of mechanism as intramolecular rejoining.

Two previous observations provide constraints on the nature of events occurring at the donor site. First, Tn*10* transposition has been shown to induce a cellular SOS response, presumably due to degradation of DNA at the donor site (ROBERTS and KLECKNER 1988). Second, previous genetic analysis suggests that transposition of an element is seldom accompanied by its "precise excision" from the donor site. [Insertion of Tn*10* or IS*10* results in duplication of a 9-bp sequence at the insertion site (KLECKNER 1979); precise excision involves restoration of the chromosome to its pre-transposon sequence by excision of the transposon sequences and one copy of the 9-bp repeat.] This conclusion was based on the observation that reversion of insertion mutations caused by Tn*10* or IS*10* occurs at a much lower frequency than transposition of the corresponding elements and that this reversion is independent of transposase expression (FOSTER *et al.* 1981b; SHEN, RALEIGH and KLECKNER 1987; J. BENDER, J. KUO and N. KLECKNER, unpublished observations).

Biochemical analysis suggests that the donor molecule is not rejoined as an integral part of the Tn*10* transposition reaction. Excision of the transposon from a circular substrate molecule by purified transposase protein *in vitro* leaves behind a linear donor DNA (HANIFORD, BENJAMIN and KLECKNER 1991; H. BENJAMIN and N. KLECKNER, personal communication).

Of the three possible fates of the transposon donor molecule, only intramolecular rejoining results in a genetically detectable alteration at the transposon do-

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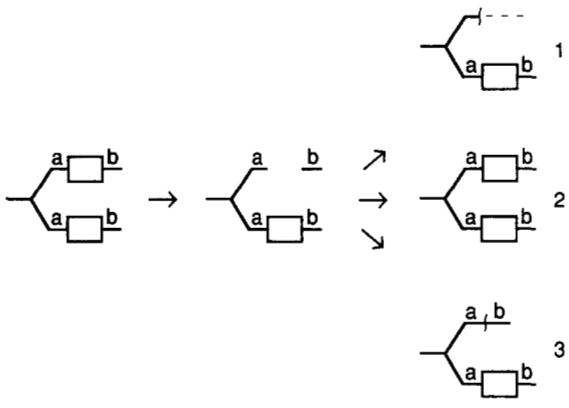

FIGURE 1.—Possible fates of the donor molecule following Tn*10* or IS*10* transposition. Fate 1, "donor suicide," results in loss of the gapped donor molecule after transposition. Fate 2 is double-strand break repair of the gapped molecule to homologous sequences on its sister chromosome, restoring the original sequence including the transposon. Fate 3 involves resealing the gapped donor molecule, possibly after some degradation at the exposed ends of DNA.

nor site. Gap restoration, by its very nature, results in reconstruction of the donor site to its parental genetic form. Similarly, if the donor chromosome is lost, the only genetically recoverable events will be interchromosomal transpositions, from one sister chromosome to another. In this case, the donor site in the recovered genome will necessarily contain unaltered parental information because it was not involved in the new transposition event.

We describe below two different genetic assays designed to detect alterations at the transposon donor site accompanying transposition. One assay compares the frequency with which an IS*10* element gives rise to "polarity relief" revertants with the frequency of transposition of the same element in the same culture. In a second assay, transpositions of an IS*10* element from one site in the bacterial genome to another are selected and the resulting isolates examined directly for alterations at the donor site. Both experiments suggest that less than 1% of IS*10* transposition events are accompanied by intramolecular rejoining of the gapped donor molecule.

MATERIALS AND METHODS

Media, enzymes and chemicals: Luria broth (LB) medium and minimal medium (M9) were as described by MILLER (1972); for solid media, 1.5% agar was added. When used, kanamycin was added to media at a concentration of 50 μg/ml, ampicillin at 100 μg/ml, tetracycline at 15 μg/ml, naladixic acid at 40 μg/ml and histidinol at 150 μg/ml. Bacteriological supplies were purchased from Difco; chemicals and antibiotics were purchased from Sigma. Standard cloning techniques were used as described by MANIATIS, FRITSCH and SAMBROOK (1982). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs.

Construction of strains used to test *hisD* polarity relief: The *Salmonella hisG*1::IS*10kan* phages used in polarity relief experiments were constructed as follows. Plasmid pNK75 (FOSTER *et al.* 1981a), an ampicillin-resistant (Amp^R) pBR322 derivative that carries the *Salmonella his* operator/promoter (*O/P*), *G* and *D* genes on an *Eco*RI fragment, was used as the target for insertions of IS*10kan* wild type (WT) from λNK1220 (*b*221 *cI*857 *cI*::IS*10kan*WT P_{am}80) or for insertions of IS*10kan*HH104 DR7 from λNK1223 (*b*221 *cI*857 *cI*::IS*10kan*HH104 DR7 P_{am}80). [The IS*10kan* constructions and the HH104 and DR7 mutations are described in ROBERTS *et al.* (1985).] To isolate transpositions from the phages into pNK75, kanamycin-resistant (Kan^R) transpositions from the phages into a strain transformed with pNK75 were pooled and plasmid DNAs were extracted. These DNAs were transformed into NK5676 (*gal*1,2$^-$ *lacZ*$^-$ Δ*his strA*549 r^-/m^+/F′*his hisG*2416$_{UGA}$) and grown on ampicillin-kanamycin medium to select Kan^R transposon insertions into the Amp^R target plasmid. Insertions in the *hisG* gene on the plasmid were screened for as *his*$^-$ auxotrophs on minimal medium. Amp^R Kan^R *his*$^-$ plasmids were restriction mapped to determine the site and orientation of transposon insertions. Isogenic *hisG*1::IS*10kan*WT (pNK1219) and IS*10kan*HH104 DR7 (pNK1221) plasmids (in the orientation shown in Figure 2) were isolated and used to construct *hisG*1::IS*10kan*HH104 (pNK1223) and IS*10kan*DR7 (pNK1225) plasmids by separating and remixing the ends of the IS*10*s via a unique *Hin*dIII site in the Tn*903 kan* gene and a unique *Bgl*II site in the upstream *hisG* DNA. Plasmid pNK2727 (IS*10kan*Δ*Nco*I) is isogenic to the IS*10kan*WT plasmid pNK1219 except that it carries an in-frame internal deletion of 300 bp between the two *Nco*I sites in the IS*10* transposase gene (HALLING *et al.* 1982).

Each of the five isogenic *hisG*1::IS*10kan* plasmid constructions was crossed on to λNK1039, a phage carrying the *Eco*RI *Salmonella his O/P G D* fragment from pNK75 in the unique *Eco*RI site (between 21,226 and 26,104 on the λ map) of λRP167 (*imm*^{21} *nin5*) (MAURER, MEYERS and PTASHNE 1980), oriented so that *hisD* gene is closest to the λ*J* gene. λNK1039 was grown on transformants of each *hisG*1::IS*10kan* plasmid, and Kan^R recombinant phages were isolated by replica plating plaques on to kanamycin medium to select Kan^R lysogens from the centers of plaques, then inducing and purifying the phages from these lysogens. The structures of the *hisG*1::IS*10kan* phage DNAs were checked by restriction mapping. The resulting five isogenic phages, carrying *hisG*1::IS*10kan*WT (λNK1249), HH104 (λNK1250), DR7 (λNK1251), HH104 DR7 (λNK1252) and Δ*Nco*I (λNK1291), were used to lysogenize NK8068 (*gal*1,2$^-$ *lacZ*$^-$ Δ*his recA*$^-$ *strA*549 r^-/m^+/pOX38::miniTn*10*Tet^R). Single lysogens were identified using a standard *ter* test (MOUSSET and THOMAS 1969).

These single lysogen strains were used to measure the frequency of Hol^+ revertants and IS*10kan* transposition. Ten to twelve independent cultures of each strain were grown to saturation in LB medium, spun down, resuspended in an equal volume of 0.85% saline, and titered on M9 minimal histidinol medium and LB medium to determine the frequency of Hol^+ revertants in each culture. The same cultures were used as donors in a conjugational "mating out" assay for transposition frequency (FOSTER *et al.* 1981a) with NK8032 (Δ*lacpro*$_{XIII}$*argE*$_{am}$ *recA*$^-$ Nal^R Rif^R) as the recipient strain.

In *recA*$^+$ strains, Hol^+ revertants arise from duplications of the *hisD* gene: The *hisD* polarity relief assay was set up in a *recA*$^-$ strain because in a *recA*$^+$ strain artifactual polarity relief occurs at a high frequency due to RecA-dependent chromosomal duplication of the *his* operon and masks the much less frequent true polarity relief due to excision of the polar IS*10kan* element. This phenomenon was investigated by constructing the IS*10kan* polarity relief

assay in the *his* operon of the *Salmonella* chromosome in a *recA*$^+$ strain.

The various *hisG*1::IS*10kan* λ phages were infected into an "Eschenella" strain, NK6912 (*Salmonella* LT-2 Δ*malB met*$^-$ *val*$^-$*trp*$^-$ *galE*$^-$ *hcm*$^-$ Str^R/F′ 112 *malB*$^+$ $_{E.coli}$) a *Salmonella* derivative that can adsorb λ. Kan^R *his*$^-$ *hisG*::IS*10kan* recombinants into the chromosomal *his* operon from these infections were isolated and transduced into the chromosome of NK80 (*Salmonella* LT-2 *edd*$^-$) with P22 *int*3 HT12/4 *c*+ +/ −*h*21*m*3.

Saturated LB cultures of the *hisG*1::IS*10kan*WT NK80 derivative, NK1607, gave Hol$^+$ revertants at a frequency of 10^{-4}. When these revertants were restreaked on minimal histidinol medium, they gave a heterogeneous population of large revertant-sized colonies and tiny background growth-sized colonies. While the large colonies continued to throw off tiny colonies after repeated restreaking, the tiny colonies were stable, only giving rise to more tiny colonies. When the large colonies were transduced to *recA*$^-$ (from NK1616 = LT-2 *srl*$^-$203::Tn*10*dCam^R *recA*1) their "large" phenotype was stabilized. These observations are all diagnostic of RecA-dependent chromosomal duplications (ANDERSON and ROTH 1978b). In fact, chromosomal duplications of the *Salmonella his* operon have been selected as Hol$^+$ revertants of a strain deleted for the *his* promoter (ANDERSON and ROTH 1978a), a situation analogous to the polarity relief assay strains, where transcription from the promoter is prematurely terminated by the IS*10* insertion.

NK1607 colonies containing *his* operon chromosomal duplications were explicitly isolated and tested for their phenotypes on minimal histidinol medium. Using the method of ANDERSON and ROTH (1981), two alleles of the *gnd* (gluconate dehydrogenase) gene that is just downstream of the *his* operon were selected for simultaneously. In an *edd*$^-$ strain background, *gnd*$^-$ *Salmonella* fail to metabolize gluconate as a carbon source and cannot grow on minimal gluconate (0.2%) medium (PEYRU and FRAENKEL 1968). NK1607 was transduced to *gnd*::Tn*10* (from NK114 = LT2 *gnd*161::Tn*10*) and Tet^R colonies were selected on minimal glucose medium. These colonies contain *gnd* gene duplications–one wild-type gene and one gene transduced to *gnd*::Tn*10* (Tet^R)–and display an unstable *gnd*$^+$ phenotype on gluconate-tetrazolium medium (KLECKNER, REICHARDT and BOTSTEIN 1979). Some of these colonies contain duplications that extend through the *his* operon as well. Indeed, when tested on minimal histidinol medium, some of the *gnd* duplication colonies displayed the unstable colony size phenotype previously observed for Hol$^+$ revertants of NK1607. Thus, unstable Hol$^+$ revertants do in fact arise from RecA-dependent chromosomal duplications. In order to eliminate this high frequency of artifactual Hol$^+$ revertants, the polarity relief assay must be performed in a *recA*$^-$ strain background.

Construction of IS*10kan-tlf*: A reversibly Kan^R derivative of IS*10kan-tlf*, IS*10kan-tlf*Kan^R, that contains direct repeats of the unexpressed Tn*5* *kan-tlf* gene and the expressed Tn*5* *kan* gene was first constructed to facilitate genetic manipulation of the element. The transposition of this element can be selected for by Kan^R without requiring fusion of the *kan-tlf* gene to an expressed gene. The expressed *kan* gene can then be removed by homologous recombination between the directly repeated *kan* genes to yield a kanamycin-sensitive IS*10kan-tlf* insertion.

The IS*10kan-tlf*Kan^R element was constructed on a plasmid as follows. The Tn*5* *kan-tlf* *Bam*HI to *Sal*I fragment from pKM109/9 (REISS, SPRENGEL and SCHALLER 1984) was cloned between the *Bcl*I site at bp 66 and the *Stu*I site (converted to *Sal*I) at bp 1022 of IS*10* (HALLING *et al.* 1982) on a derivative of the Amp^R IS*10* plasmid pNK82 (SIMONS and KLECKNER 1983) that carries four tandem repeats of the *rrnB* operon ribosomal RNA terminators (BROSIUS *et al.* 1981) upstream of the outer end of IS*10* (pNK2335) to make pNK1695. The *Bcl*I/*Bam*HI fusion of this plasmid will provide an open reading frame through the IS*10* end into *kan-tlf* if the element inserts into an expressed gene so that bp 1 of IS*10* is in frame with the expressed gene's reading frame. The direct repeat *kan* gene was cloned downstream of the *kan-tlf* gene on pNK1695 at the *Sal*I site using a *Hin*dIII (converted to *Sal*I) to *Sal*I (converted to *Eco*RI) fragment from Tn*5* (ROTHSTEIN *et al.* 1980), followed by a fusion of the strong promoter P*tac* to the IS*10* transposase gene on an *Eco*RI to *Cla*I fragment from pNK474 (WAY *et al.* 1984) which substitutes for the truncated *Stu*I IS*10* end on pNK1695 a transposase gene and IS*10* end that will promote transposition of the element. This plasmid, pNK1698, thus carries the *rrnB* terminators, the outermost 66 bp of IS*10* fused to *kan-tlf*, the expressed *kan* gene, P*tac* fused to the IS*10* transposase gene through the innermost end of the element, all on an Amp^R pBR322-derived plasmid backbone.

Transpositions of IS*10kan-tlf*Kan^R off of pNK1698 on to λNK111 (*b*616 *b*519 *cI*857) were isolated by growing λNK111 on a C600 strain transformed with pNK1698, then infecting the phage stock into NK8032 and selecting for Kan^R colonies at 30°. These Kan^R lysogen colonies were screened for the T4*rII*-sensitive phenotype diagnostic of *rex*$^-$ mutations by cross-streaking against T4*rII*. Two T4*rII*-sensitive lysogens were isolated and their phages, λNK1287 and λNK1289, were induced and purified. These phages were grown on a C600 (*recA*$^+$) strain, then lysogenized in C600 and screened for Kan^S lysogens that arise from the Kan^R phages by homologous recombination. Kan^S phages, λNK1288 from λNK1287 and λNK1290 from λNK1289, were induced and purified. The *Bgl*II *rex* fragments from λNK1288 and λNK1290 were cloned and the exact positions of the IS*10kan-tlf* inserts in these fragments were determined by sequencing using a primer that reads out from the outer end of IS*10*. λNK1288 contains the IS*10kan-tlf* insert at bp 36627 to 36635 of the λ map oriented so that the *kan-tlf* gene would be transcribed in the opposite direction from the *rexA* gene (see Figure 3). This phage was lysogenized into NK5019 (*Escherichia coli* W3110) to create IS*10kan-tlf* transposition assay strain. The strain was verified to contain a single lysogen by Southern blot analysis. The second phage, λNK1290, contains the IS*10kan-tlf* insert at bp 36672 to 36680 of the λ map oriented so that the *kan-tlf* gene would be transcribed in the same direction as the *rexA* gene. This phage was also lysogenized into NK5019 and served as a negative control for hybridization studies of the structure at the donor site (described below).

Isolation and analysis of Kan^R transpositions of IS*10kan-tlf*: Hundreds of independent Kan^R transpositions in NK5019(λNK1288) were isolated as follows. The strain was titered for single colonies on minimal medium with glycerol as a carbon source and grown at 30° to maintain the *cI*857 lysogen. Under these conditions, the strain grows very slowly and individual cells contain a minimum number of chromosomes. Transpositions that occur during this growth are thus more likely to insert into the same chromosome from which they excised than if the strain were growing rapidly under rich conditions. Kan^R transpositions that arise within each titered colony were selected for by streaking each colony on LB kanamycin medium. Each streak contained 0–100 Kan^R colonies. One Kan^R colony was picked from each of 1088 of these streaks. The resulting 1088 independent Kan^R colonies were patched onto two

sets of LB plates: one set was grown at 42° and one set was grown at 30°.

Kan^R colonies were patched and grown at 42° to test for the temperature sensitive *cI*857 phenotype. The parental strain NK5019(λNK1288) was included as a positive control and the non-lysogen strain NK5019 was included as a negative control. All of the 1088 Kan^R colonies tested retained the parental temperature-sensitive phenotype.

Kan^R colonies were patched and grown at 30° to be probed for the parental *rexA*::IS*10kan-tlf* junction with an oligonucleotide of the sequence 5′ AGCTTGGCTCTGATGAAT 3′ ("IS*10*Rex" by Operon Technologies). The parental strain NK5019(λNK1288) was included as a positive control and the strain NK5019(λNK1290) (IS*10kan-tlf* inserted elsewhere in *rexA*) was included as a negative control. IS*10*Rex spans 9 bp of *rexA* (bp 36627–36635 on the λ map) and 9 bp of the outermost end of IS*10* present on λNK1288. Under conditions of appropriate stringency, this probe will hybridize to DNA with the complete complementary sequence, but not to DNA with just the *rexA* sequence, with just the IS*10* sequence, or with IS*10* inserted into a different site in *rexA*.

GeneScreen filters were laid over the plates to transfer the patches to the filters. The patches were lysed and their DNAs bound to the filters by autoclaving for 3 min. The filters were washed, prehybridized, and hybridized with the ^{32}P-labeled IS*10*Rex probe by the method of AUSUBEL *et al.* (1987). After overnight hybridization, the filters were washed at room temperature with 6 × SSC + 0.05% sodium pyrophosphate (PPi) and autoradiographed to confirm the presence of DNA from each original patch of cells. The filters were then washed at 50° in 6 × SSC + 0.05% PPi to remove all partially bound probe and autoradiographed again. Under these conditions, probe washed off the negative control DNAs but not off the positive control DNAs or the 1088 experimental DNAs (see Figure 4).

RESULTS

Measurement of *hisD* polarity relief and transposition frequencies for polar IS*10* insertions in the *Salmonella hisG* gene: The first assay will detect many but not all types of alterations at the transposon donor site. This assay involves an IS*10* insertion which is located in the first gene of an operon and which causes transcription of the operon to terminate before it reaches genes located downstream of the insertion. Derivatives that have lost the polar effect of the insertion can be selected by requiring restoration of function of a downstream gene as described below. Restoration of the wild type sequence at the donor site is not required for relief of polarity; donor chromosomes which retain all or part of both 9-bp repeat sequences or which have undergone some degradation around the original gap will be recovered. However, some types of rejoined chromosomes will not be recovered, including those which have suffered degradation of the downstream reporter gene or inappropriate "out of frame" rejoining within the first gene which leads to Rho-dependent transcription termination within the *hisG* gene (CIAMPI and ROTH 1988).

A possible relationship between reversion to polarity relief and transposition was investigated by comparing the frequency of polarity relief revertants and the frequency of IS*10* transposition in a series of isogenic strains exhibiting a wide range of IS*10* transposition frequencies.

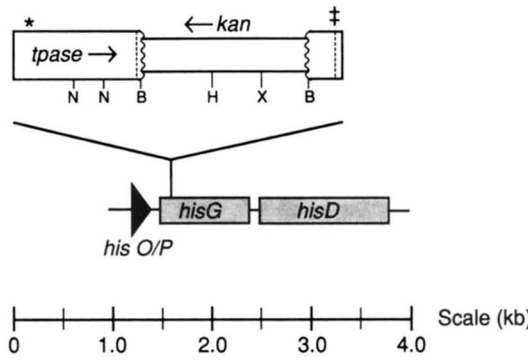

FIGURE 2.—Structure of the polar IS*10kan* insertion in the *Salmonella hisG* gene. The structure, position and orientation of the polar IS*10kan* insertions used in the *hisD* polarity relief assay are shown. The * marks the position of the HH104 transposase promoter up mutation and the ‡ marks the position of the DR7 mutation that activates the inner terminus of the IS*10kan* element. The IS*10kan* element contains direct repeats of approximately 300 bp of IS*10* sequences [from the *Stu*I site at bp 1022 to the *Xho*II site at bp 1319 (HALLING *et al.* 1982)] flanking the *kan* gene. The positions of the transposase stop codons in the repeated material (at bp 1314–1316 of IS*10*) are marked by dashed vertical lines. B = *Bam*HI site, H = *Hin*dIII site, N = *Nco*I site, and X = *Xho*I site.

The specific assay involves strains carrying an IS*10* insertion in the first gene of the *Salmonella* histidine operon, *hisG*, at a specific preferred insertion site, *hisG*1 (KLECKNER, REICHARDT and BOTSTEIN 1979; HALLING and KLECKNER 1982). The IS*10* element is marked with a *kan* gene from Tn*903* (IS*10kan*, ROBERTS *et al.* 1985) which both increases the polarity of the element and facilitates its genetic manipulation. The structure of this *hisG*1::IS*10kan* insertion is shown in Figure 2. Five isogenic variants of this insertion were analyzed: the wild type (WT) IS*10kan* element, a transposition defective transposase deletion variant (Δ*Nco*I), a variant with a single base change in the transposase promoter that increases gene expression and transposition about 100-fold (HH104, marked by * in Figure 2; FOSTER *et al.* 1981a; SIMONS *et al.* 1983), a variant with a single base change at the end of the element distal to the transposase promoter that removes a Dam methylation site and slightly increases the frequency of transposition in Dam$^+$ cells (DR7, marked by ‡ in Figure 2; ROBERTS *et al.* 1985) and a HH104 DR7 double mutant variant (ROBERTS *et al.* 1985).

IS*10* rather than Tn*10* was used for this assay because IS*10* transposes at a higher frequency and is therefore the more biologically relevant element (SHEN, RALEIGH and KLECKNER 1987; MORISATO *et al.* 1983). Also, IS*10* is superior to Tn*10* for two technical reasons. First, the simple IS*10* element does not give rise to certain transposition-related DNA

rearrangements, deletions and deletion/inversions, that Tn*10* promotes from its internal IS*10* ends (KLECKNER, REICHARDT and BOTSTEIN 1979). Second, IS*10* is less likely to promote precise excisions and related "nearly precise" excisions than is Tn*10* since it lacks a perfect inverted symmetry at its ends (FOSTER *et al.* 1981b).

Relief of polarity was examined using the second gene in the histidine operon, *hisD*, which encodes histidinol dehydrogenase, the last enzyme in the histidine biosynthesis pathway. Because this enzyme converts histidinol to histidine, *hisG*$^-$ *hisD*$^+$ polarity relief revertants can be selected by their ability to grow on minimal medium supplemented with histidinol (KLECKNER *et al.* 1975). Thus, derivatives in which the polarity of IS*10kan* on *hisD* has been relieved can be selected as "Hol$^+$" revertants without requiring restoration of an intact *hisG* gene at the transposon donor site.

Transposition was assayed in the same cultures examined for polarity relief by the conjugational "mating-out" assay (FOSTER *et al.* 1981a). The host strains carried a pOX38 episome marked with tetracycline resistance (pOX38::miniTn*10*Tet^R). The frequency of IS*10kan* transpositions into the episome is determined after mating with a suitable donor strain as the ratio of Kan^R Tet^R exconjugants to total Tet^R exconjugants. Transpositions into pXO38 represent about 10% of all transpositions in the cell for IS*10*-derived elements (N. KLECKNER, data not shown), so the total transposition frequency for a culture is calculated as ten times the frequency of transpositions into the episome.

To generate the strains for this analysis, the five *hisG*1::IS*10kan* variants were constructed on plasmids and crossed by genetic recombination onto a lambda derivative carrying the *Salmonella his* operator/promoter (O/P) and the *hisG* and *hisD* genes (see MATERIALS AND METHODS). Single lysogens of these phages were then isolated in a *recA*$^-$ *E. coli* strain carrying a complete deletion of the histidine operon and pOX38::miniTn*10*Tet^R (NK8068).

Ten to twelve independent cultures of each lysogen were examined in parallel for the frequency of Hol$^+$ revertants and for the frequency of IS*10kan* transposition. In the NK8068 host strain, the *hisD* prophage expresses only enough histidinol dehydrogenase to allow growth of tiny background colonies on minimal histidinol medium, while events that remove the polar IS*10kan* effect yield fast-growing Hol$^+$ revertant colonies. For the mating out assay, each culture was mated with a Nal^R recipient strain (NK8032). Total exconjugants were selected as Nal^R Tet^R, and exconjugants carrying IS*10kan* insertions in the episome were selected as Nal^R Kan^R.

The frequencies of Hol$^+$ reversion and Kan^R transposition for each IS*10kan* variant are shown in Table 1. For wild-type IS*10kan*, the frequency of transpositions is about 125-fold higher than the frequency of Hol$^+$ revertants. For the extremely active IS*10kan*HH104DR7 variant, the frequency of transpositions is about 3000-fold higher than the frequency of Hol$^+$ revertants. These data suggest that the majority of IS*10* transposition events do not result in relief of polarity. In fact, most of the polarity relief events detected arise independently of IS*10* transposition: the IS*10kan*HH104DR7 element transposes more than 1000 times more frequently than the defective IS*10kan*Δ*Nco*I element, but is increased for generation of polarity relief revertants by less than twofold.

Examination of the transposon donor site after IS*10kan-tlf* transposition out of a lambda prophage *rex* gene: Because the polarity relief assay might not detect certain resealed donor molecule structures, we developed a second genetic assay for the fate of the IS*10* donor molecule which could recover most possible intramolecular rejoining events. This second approach to analysis of donor site structure involves a special IS*10* construct which can be used to select transpositions from one site in the bacterial genome to another. This transposon construct contains a Tn*5* *kan* gene which lacks appropriate translational and transcriptional start signals but which can be activated by insertion of the element into an actively expressed gene in the appropriate orientation and reading frame. We call this element IS*10kan-tlf* to distinguish it from the IS*10kan* elements used in the experiments described in the previous section. If one starts with a cell carrying the IS*10kan-tlf* construct in an unexpressed context, transpositions of the element to new locations can be obtained in a single step by selecting for kanamycin resistance. Such Kan^R derivatives can then be examined for the genetic and physical structure of the original transposon donor site. Transposition events selected in this way might have arisen by either intra- or interchromosomal transposition. As explained above, intermolecular events will necessarily carry an unaltered donor site. The proportion of interchromosomal events was minimized in these experiments by isolating transposition events from cells grown under conditions where the average number of chromosomes per cell is expected to be less than two (minimal glycerol medium; see MATERIALS AND METHODS). If transposition results in efficient intramolecular rejoining of the donor chromosome, under these conditions at least half of the Kan^R clones examined should have resulted from intrachromosomal transposition.

The strain constructed for this analysis was wild-type *E. coli* (NK5019 = W3110) carrying the IS*10kan-tlf* construct in the *rexA* gene of a single lambda

TABLE 1

Frequencies of Hol$^+$ reversion and of transposition for polar IS*10kan* insertions in *hisG*

IS*10kan* construction	(*A*) Frequency Hol$^+$ revertants	(*B*) Frequency transposition	(*B*)/(*A*)
Δ*Nco*I (tpase Δ, ends WT)	2.36×10^{-7}	$<0.1 \times 10^{-5}$	NA
WT (tpase WT, ends WT)	2.08×10^{-7}	2.6×10^{-5}	125
DR7 (tpase WT, Me$^-$ inside end)	1.78×10^{-7}	3.8×10^{-5}	213
HH104 (tpase up, ends WT)	3.67×10^{-7}	62.8×10^{-5}	1710
HH104 DR7 (tpase up, Me$^-$ inside end)	3.63×10^{-7}	112.2×10^{-5}	3090

Isogenic phages carrying the IS*10kan* derivatives listed inserted in the *Salmonella hisG* gene and polar on the *hisD* gene (as shown in Figure 2) were lysogenized in NK8068. Cultures of each lysogen were titered for Hol$^+$ revertants on minimal histidinol medium and used as donors in a conjugational "mating out" assay for transposition frequency. Numbers represent the average of 10–12 independent cultures of each strain. Transposition frequencies measured as Kan^R exconjugants per total (Tet^R) exconjugants only detect transpositions into the pOX38::Tet^R element in NK8068, which represent only about 10% of total transpositions in the strain for IS*10*-derived elements. These numbers are therefore multiplied by 10 to convert them to total transposition frequencies.

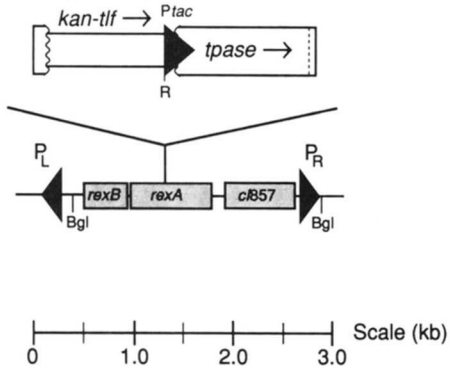

FIGURE 3.—Structure of the IS*10kan-tlf* insertion in the λ *rexA* gene. The structure, position and orientation of the IS*10kan-tlf* insertion in the *rexA* gene of λNK1288 are shown. The position of the transposase stop codon is marked by a dashed vertical line. Bgl = *Bgl*II site, R = *Eco*RI site.

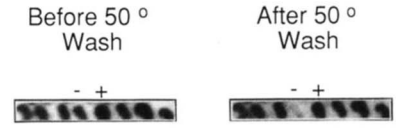

FIGURE 4.—Hybridization of the IS*10*Rex probe to Kan^R transposition derivatives of NK5019(λNK1288) before and after a stringent wash. The left panel shows an autoradiogram of Kan^R transposition derivatives of NK5019(λNK1288) after colony hybridization to the kinased probe IS*10*Rex and a nonstringent room temperature wash. The sample marked (−) is the negative control strain NK5019(λNK1290) and the sample marked (+) is the positive parental strain NK5019(λNK1288). The right panel shows the same samples after a stringent wash at 50°.

prophage carrying the thermosensitive repressor mutation *cI*857 (λNK1288; Figure 3). The *rexA*::IS*10kan-tlf* insertion is oriented such that the *kan-tlf* gene would be transcribed in the opposite direction from *rexA*. The structure of the prophage in the lysogen was verified by Southern blot analysis (data not shown).

As described in MATERIALS AND METHODS, 1088 independent Kan^R derivatives of NK5019(λNK1288) were isolated and patched onto two sets of LB plates which were then probed by genetic and physical methods for the integrity of the transposon donor site. The first set of plates was grown at 42° to assay for survival following prophage induction. All 1088 derivatives exhibited the same temperature sensitive phenotype as the parental strain. This result indicates that prophage genes in the immunity region are still present; deletions extending from the *rexA* gene outward through the P_L and P_R promoters would have rendered the prophage insensitive to temperature treatment (EISEN *et al.* 1970).

The second set of plates were grown at 30° and replica plated onto GeneScreen filters which were used to probe for the integrity of one of the *rexA*/IS*10kan-tlf* junction sequences. Filters were subjected to colony hybridization with an 18-nucleotide oligonucleotide probe spanning the junction between the outer terminus of IS*10* and flanking *rexA* sequences. When hybridization is carried out at an appropriate stringency, this probe hybridizes only to DNAs with exactly the parental donor site structure (Figure 4). All 1088 Kan^R derivatives exhibited efficient hybridization to this probe. This result indicates that all of these derivatives retain the critical junction sequence.

Taken together, these results suggest that all 1088 derivatives retain the original parental *rexA*::IS*10kan-tlf* sequences intact at the transposon donor site. Correcting for the possibility that half of these events might represent intermolecular transpositions, these results indicate that intramolecular rejoining occurred in fewer than 1/544 independent cases, or less than about 0.2%.

DISCUSSION

Tn*10* transposition involves efficient double strand excision of the transposon from the donor site. This excision leaves behind a gap in the donor DNA. Previous results have shown that Tn*10* transposition induces SOS functions, implying that there is extensive degradation of donor DNA after transposon excision (ROBERTS and KLECKNER 1988). Previous results have also shown that Tn*10* and IS*10* transpositions yield neither efficient transposase-promoted

rejoining of the donor site nor genetically "precise excision" of transposon sequences plus one copy of the 9-bp repeat sequence to regenerate a wild-type donor gene (HANIFORD, BENJAMIN and KLECKNER 1991; H. BENJAMIN and N. KLECKNER, PERSONAL COMMUNICATION; FOSTER *et al.* 1981b; SHEN, RALEIGH and KLECKNER 1987).

The data presented here suggest that IS*10* transposition also does not result in host-promoted intramolecular rejoining of the interrupted donor chromosome, with or without deletion of sequences around the donor site. The strongest evidence against intramolecular rejoining is provided by the second assay described: fewer than 0.2% of intrachromosomal transposition events result in any genetically detectable alteration at the donor site. Supporting evidence is provided by the first assay described: the frequency of IS*10kan*HH104DR7 transposition is 3000 times higher than the frequency with which this element gives rise to polarity relief revertants.

Of the two assays the polarity relief assay yields the less definitive results because many but not all possible intramolecular rejoining events would be detected by this test. Of particular concern is the possibility that the donor site is subject to substantial DNA degradation prior to rejoining; such degradation might frequently result in deletion of the *hisD* reporter gene located just 200 bp away from the donor site, and is particularly likely in the *recA*$^-$ strain background required by these experiments. The IS*10kan-tlf* activation assay is not subject to this or any other limitation except for the correction needed to take into account the possibility of intermolecular transposition events.

These experiments do not distinguish between the two remaining possible fates of the donor molecule, donor suicide and gap reconstruction. In either of these cases, as discussed above, all genetically recoverable chromosomes that have undergone transposition events are predicted to retain the original parental structure at the donor site.

Previous observations on Tn*10* transposition are compatible with both of these possible fates. Induction of SOS functions by Tn*10* transposition could result from either complete degradation of the donor chromosome in the case of donor suicide or, in the case of gap reconstruction, degradation around the site of transposon excision prior to interaction of the broken chromosome with an intact sister chromosome. Furthermore, in both cases, recovery of viable cells that contain a transposition requires that at least two copies of the donor region of the chromosome be present in the cell at the time the transposition event occurs. IS*10* transposition is regulated by Dam methylation in a way that should ensure that this requirement is met. IS*10* is active only when it is appropriately hemimethylated. As a consequence, transposition is predicted to occur immediately after passage of the chromosomal replication fork and to involve only one of the two newly generated hemimethylated species present on the two new sister molecules (ROBERTS *et al.* 1985).

Previous observations on the biology of *E. coli* are also compatible with both possible donor molecule fates. Presumably, bacteria can survive complete degradation of an entire chromosome as long as the cell contains more than one chromosome. Also, several lines of evidence suggest that recombinational gap reconstruction events can occur in *E. coli* (THALER and STAHL 1988). An intriguing possibility is that the ends of the flanking donor DNA are bound by transposase protein in such a way as to facilitate double-strand break repair recombination.

It will be interesting to compare the Tn*10* donor molecule fate to the donor molecule fates of other transposons that transpose by a nonreplicative mechanism. Recent experiments have suggested that *P* element transposition in Drosophila results in efficient concomitant gap reconstruction of the transposon donor site (ENGLES *et al.* 1990). In heterozygous Drosophila strains carrying a *P* element insertion on one chromosome and wild-type information at the same site on the other chromosome, transposase-dependent asymmetric gene conversion of the *P* element donor site to wild-type sequences is observed. This gene conversion is interpreted to result from excision of the transposon during nonreplicative transposition followed by gap reconstruction of the transposon donor site, with the heterologous wild-type information on the other chromosome serving as template. In contrast, many plant transposons appear to undergo nonreplicative transposition accompanied by efficient donor site resealing which creates small deletions or rearrangements of sequences immediately adjacent to the transposon donor site (COEN *et al.* 1989; FEDOROFF 1989). The ability of these plant transposons to yield donor site resealing might be a direct consequence of their transposition mechanism or it might reflect the ability of the plant cell to repair linearized DNA molecules after transposition.

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